FIGURE 1[a]

(SEQ ID NO:1-nucleotide sequence and SEQ ID NO:2-amino acid sequence)

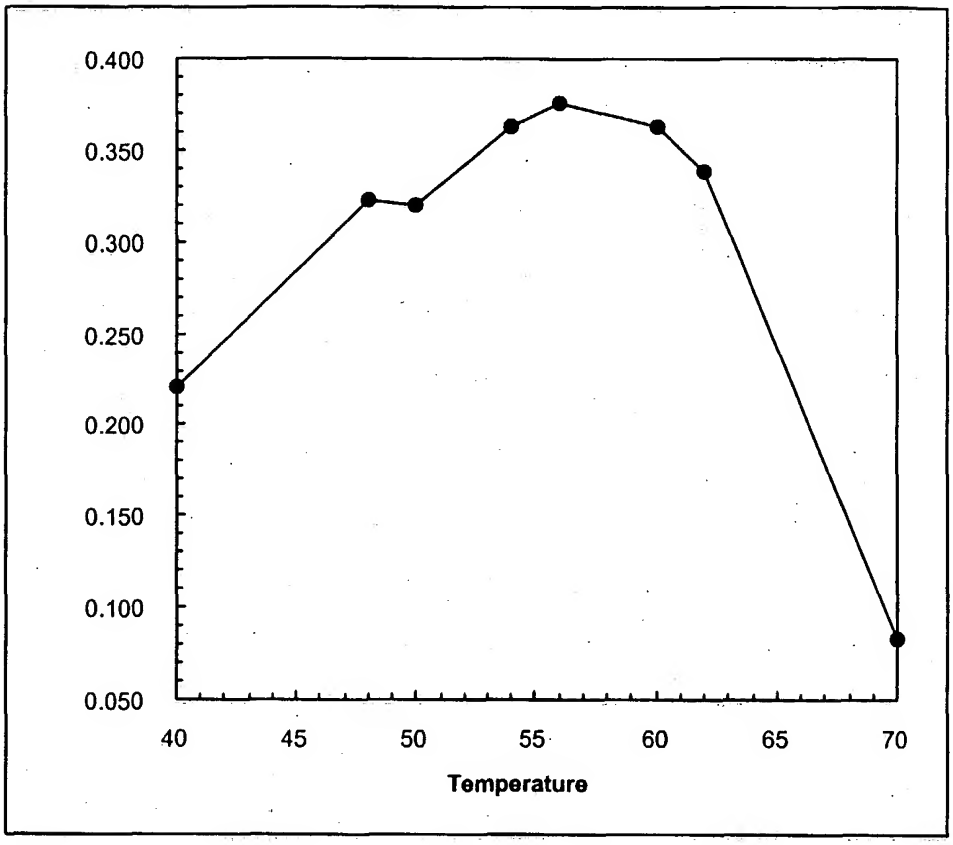
***Escherichia coli* B Phytase Sequence**

```
1
ATG AAA GCG ATC TTA ATC CCA TTT TTA TCT CTT CTG ATT CCG TTA ACC CCG
Met Lys Ala Ile Leu Ile Pro Phe Leu Ser Leu Leu Ile Pro Leu Thr Pro

CAA TCT GCA TTC GCT CAG AGT GAG CCG GAG CTG AAG CTG GAA AGT GTG GTG
Gln Ser Ala Phe Ala Gln Ser Glu Pro Glu Leu Lys Leu Glu Ser Val Val

ATT GTC AGT CGT CAT GGT GTG CGT GCT CCA ACC AAG GCC ACG CAA CTG ATG
Ile Val Ser Arg His Gly Val Arg Ala Pro Thr Lys Ala Thr Gln Leu Met

CAG GAT GTC ACC CCA GAC GCA TGG CCA ACC TGG CCG GTA AAA CTG GGT TGG
Gln Asp Val Thr Pro Asp Ala Trp Pro Thr Trp Pro Val Lys Leu Gly Trp

CTG ACA CCG CGN GGT GGT GAG CTA ATC GCC TAT CTC GGA CAT TAC CAA CGC
Leu Thr Pro Arg Gly Gly Glu Leu Ile Ala Tyr Leu Gly His Tyr Gln Arg

CAG CGT CTG GTA GCC GAC GGA TTG CTG GCG AAA AAG GGC TGC CCG CAG TCT
Gln Arg Leu Val Ala Asp Gly Leu Leu Ala Lys Lys Gly Cys Pro Gln Ser

GGT CAG GTC GCG ATT ATT GCT GAT GTC GAC GAG CGT ACC CGT AAA ACA GGC
Gly Gln Val Ala Ile Ile Ala Asp Val Asp Glu Arg Thr Arg Lys Thr Gly

GAA GCC TTC GCC GCC GGG CTG GCA CCT GAC TGT GCA ATA ACC GTA CAT ACC
Glu Ala Phe Ala Ala Gly Leu Ala Pro Asp Cys Ala Ile Thr Val His Thr

CAG GCA GAT ACG TCC AGT CCC GAT CCG TTA TTT AAT CCT CTA AAA ACT GGC
Gln Ala Asp Thr Ser Ser Pro Asp Pro Leu Phe Asn Pro Leu Lys Thr Gly

GTT TGC CAA CTG GAT AAC GCG AAC GTG ACT GAC GCG ATC CTC AGC AGG GCA
Val Cys Gln Leu Asp Asn Ala Asn Val Thr Asp Ala Ile Leu Ser Arg Ala

GGA GGG TCA ATT GCT GAC TTT ACC GGG CAT CGG CAA ACG GCG TTT CGC GAA
Gly Gly Ser Ile Ala Asp Phe Thr Gly His Arg Gln Thr Ala Phe Arg Glu

CTG GAA CGG GTG CTT AAT TTT CCG CAA TCA AAC TTG TGC CTT AAA CGT GAG
Leu Glu Arg Val Leu Asn Phe Pro Gln Ser Asn Leu Cys Leu Lys Arg Glu

AAA CAG GAC GAA AGC TGT TCA TTA ACG CAG GCA TTA CCA TCG GAA CTC AAG
Lys Gln Asp Glu Ser Cys Ser Leu Thr Gln Ala Leu Pro Ser Glu Leu Lys

GTG AGC GCC GAC AAT GTC TCA TTA ACC GGT GCG GTA AGC CTC GCA TCA ATG
Val Ser Ala Asp Asn Val Ser Leu Thr Gly Ala Val Ser Leu Ala Ser Met

CTG ACG GAG ATA TTT CTC CTG CAA CAA GCA CAG GGA ATG CCG GAG CCG GGG
Leu Thr Glu Ile Phe Leu Leu Gln Gln Ala Gln Gly Met Pro Glu Pro Gly

TGG GGA AGG ATC ACC GAT TCA CAC CAG TGG AAC ACC TTG CTA AGT TTG CAT
Trp Gly Arg Ile Thr Asp Ser His Gln Trp Asn Thr Leu Leu Ser Leu His

AAC GCG CAA TTT TAT TTG CTA CAA CGC ACG CCA GAG GTT GCC CGC AGC CGC
Asn Ala Gln Phe Tyr Leu Leu Gln Arg Thr Pro Glu Val Ala Arg Ser Arg

GCC ACC CCG TTA TTG GAT TTG ATC ATG GCA GCG TTG ACG CCC CAT CCA CCG
Ala Thr Pro Leu Leu Asp Leu Ile Met Ala Ala Leu Thr Pro His Pro Pro
```

FIGURE 1b

```
CAA AAA CAG GCG TAT GGT GTG ACA TTA CCC ACT TCA GTA CTG TTT ATT GCC
Gln Lys Gln Ala Tyr Gly Val Thr Leu Pro Thr Ser Val Leu Phe Ile Ala

GGA CAC GAT ACT AAT CTG GCA AAT CTC GGC GGC GCA CTG GAG CTC AAC TGG
Gly His Asp Thr Asn Leu Ala Asn Leu Gly Gly Ala Leu Glu Leu Asn Trp

ACG CTT CCC GGT CAG CCG GAT AAC ACG CCG CCA GGT GGT GAA CTG GTG TTT
Thr Leu Pro Gly Gln Pro Asp Asn Thr Pro Pro Gly Gly Glu Leu Val Phe

GAA CGC TGG CGT CGG CTA AGC GAT AAC AGC CAG TGG ATT CAG GTT TCG CTG
Glu Arg Trp Arg Arg Leu Ser Asp Asn Ser Gln Trp Ile Gln Val Ser Leu

GTC TTC CAG ACT TTA CAG CAG ATG CGT GAT AAA ACG CCG CTG TCA TTA AAT
Val Phe Gln Thr Leu Gln Gln Met Arg Asp Lys Thr Pro Leu Ser Leu Asn

ACG CCG CCC GGA GAG GTG AAA CTG ACC CTG GCA GGA TGT GAA GAG CGA AAT
Thr Pro Pro Gly Glu Val Lys Leu Thr Leu Ala Gly Cys Glu Glu Arg Asn

GCG CAG GGC ATG TGT TCG TTG GCA GGT TTT ACG CAA ATC GTG AAT GAA GCA
Ala Gln Gly Met Cys Ser Leu Ala Gly Phe Thr Gln Ile Val Asn Glu Ala

CGC ATA CCG GCG TGC AGT TTG AGA TCT CAT CAC CAT CAC CAT CAC TAA  1323
Arg Ile Pro Ala Cys Ser Leu Arg Ser His His His His His His End
```

FIGURE 2
pH/Temperature Profile and Stability



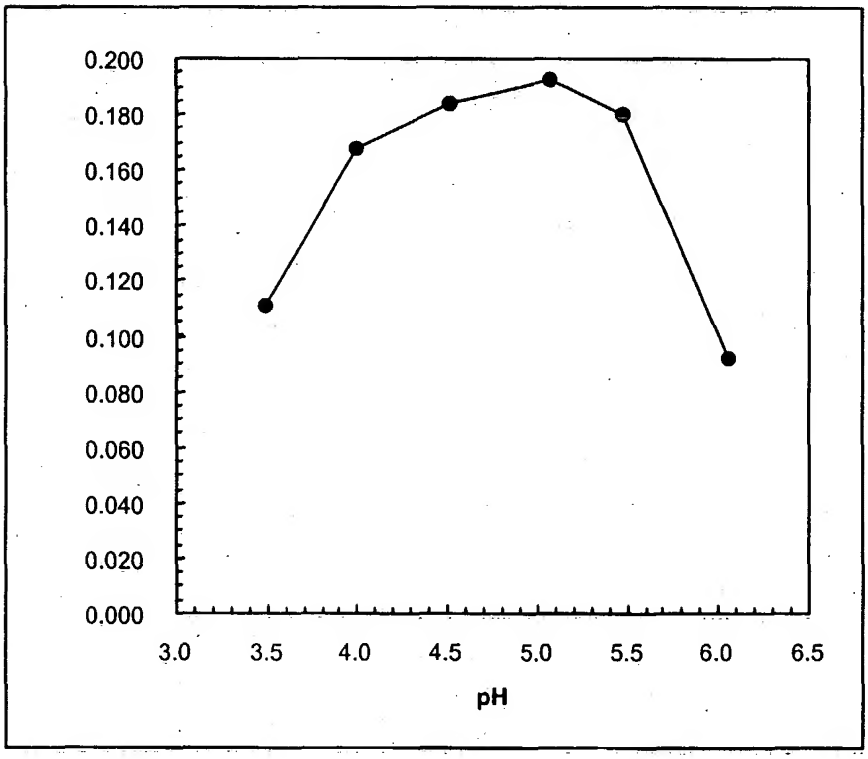


Figure 3:

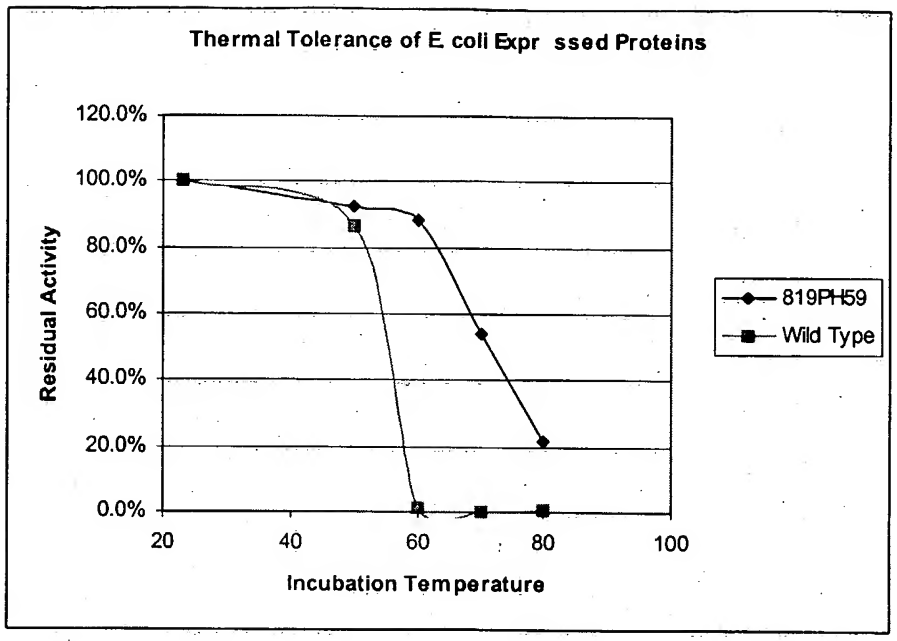

Thermal Tolerance of E. coli Expr ssed Proteins
120.0%
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
Residual Activity
20
40
60
80
100
Incubation Temperature
819PH59
Wild Type

Figure 4

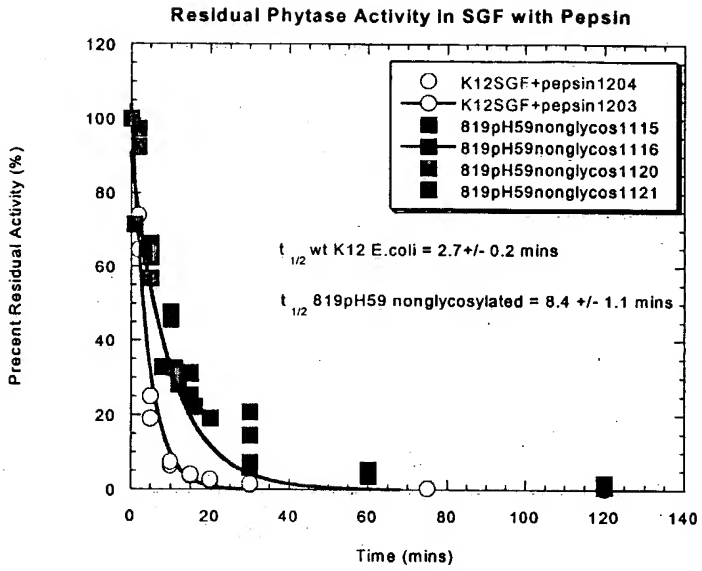

Residual Phytase Activity in SGF with Pepsin
Precent Residual Activity (%)
Time (mins)
K12SGF+pepsin1204
K12SGF+pepsin1203
819pH59nonglycos1115
819pH59nonglycos1116
819pH59nonglycos1120
819pH59nonglycos1121
t 1/2 wt K12 E.coli = 2.7+/- 0.2 mins
t 1/2 819pH59 nonglycosylated = 8.4 +/- 1.1 mins
0
20
40
60
80
100
120
140

Figure 5:

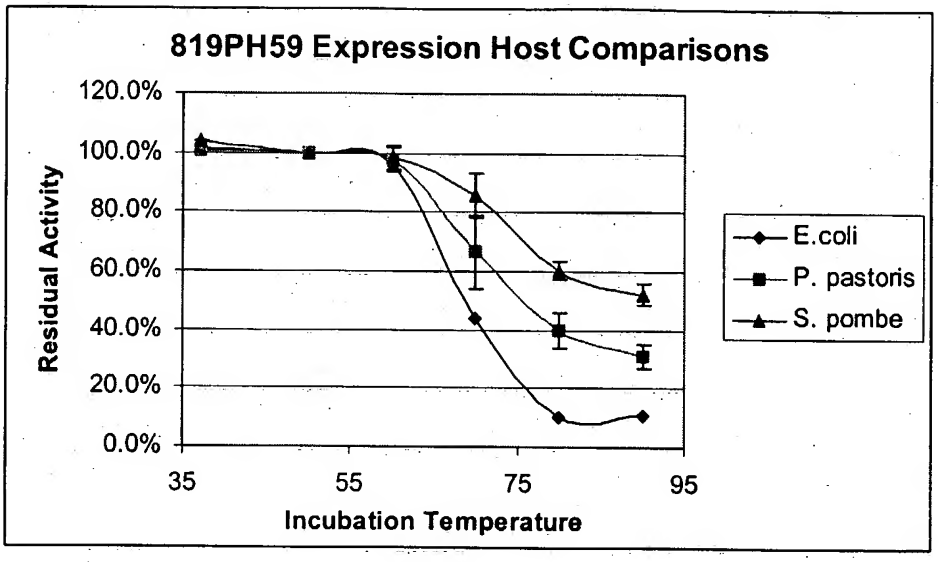

819PH59 Expression Host Comparisons
120.0%
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
Residual Activity
35
55
75
95
Incubation Temperature
E.coli
P. pastoris
S. pombe

Figure 6.

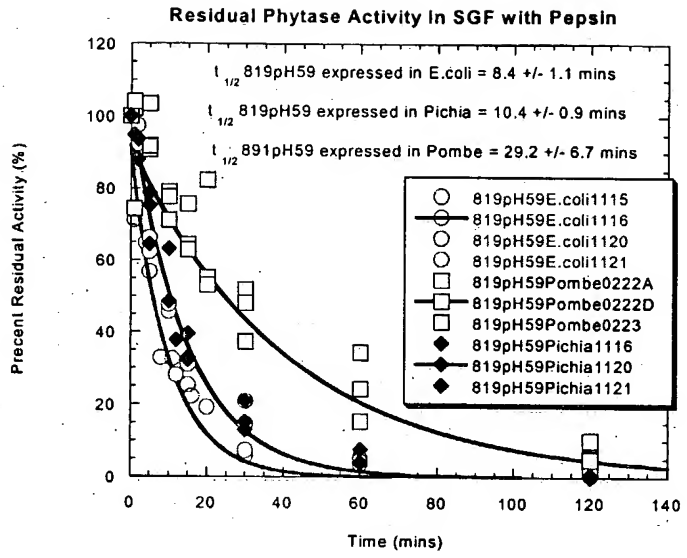

Residual Phytase Activity In SGF with Pepsin
t 1/2 819pH59 expressed in E.coli = 8.4 +/- 1.1 mins
t 1/2 819pH59 expressed in Pichia = 10.4 +/- 0.9 mins
t 1/2 891pH59 expressed in Pombe = 29.2 +/- 6.7 mins
819pH59E.coli1115
819pH59E.coli1116
819pH59E.coli1120
819pH59E.coli1121
819pH59Pombe0222A
819pH59Pombe0222D
819pH59Pombe0223
819pH59Pichia1116
819pH59Pichia1120
819pH59Pichia1121
Precent Residual Activity (%)
Time (mins)
0
20
40
60
80
100
120
140

Figure 7a

E. coli appA (GenBank accession no. M58708) (SEQ ID NO:7)

1 taaggagcag aaacaatgtg gtatttactt tggttcgtcg gcattttgtt gatgtgttcg
61 ctctccaccc ttgtgttggt atggctggac ccgcgtctga aaagttaacg aacgtaggcc
121 tgatgcggcg cattagcatc gcatcaggca atcaataatg tcagatatga aaagcggaaa
181 catatcgatg aaagcgatct taatcccatt tttatctctt ctgattccgt taaccccgca
241 atctgcattc gctcagagtg agccggagct gaagctggaa agtgtggtga ttgtcagtcg
301 tcatggtgtg cgtgctccaa ccaaggccac gcaactgatg caggatgtca ccccagacgc
361 atggccaacc tggccggtaa aactgggttg gctgacaccg cgnggtggtg agctaatcgc
421 ctatctcgga cattaccaac gccagcgtct ggtagccgac ggattgctgg cgaaaaaggg
481 ctgcccgcag tctggtcagg tcgcgattat tgctgatgtc gacgagcgta cccgtaaaac
541 aggcgaagcc ttcgccgccg ggctggcacc tgactgtgca ataaccgtac atacccaggc
601 agatacgtcc agtcccgatc cgttatttaa tcctctaaaa actggcgttt gccaactgga
661 taacgcgaac gtgactgacg cgatcctcag cagggcagga gggtcaattg ctgactttac
721 cgggcatcgg caaacggcgt ttcgcgaact ggaacgggtg cttaatttte cgcaatcaaa
781 cttgtgcctt aaacgtgaga aacaggacga aagctgttca ttaacgcagg cattaccatc
841 ggaactcaag gtgagcgccg acaatgtctc attaaccggt gcggtaagcc tcgcatcaat
901 gctgacggag atatttctcc tgcaacaagc acagggaatg ccggagccgg ggtggggaag
961 gatcaccgat tcacaccagt ggaacacctt gctaagtttg cataacgcgc aattttattt
1021 gctacaacgc acgccagagg ttgcccgcag ccgcgccacc ccgttattag atttgatcaa
1081 gacagcgttg acgccccatc caccgcaaaa acaggcgtat ggtgtgacat tacccacttc
1141 agtgctgttt atcgccggac acgatactaa tctggcaaat ctcggcggcg cactggagct
1201 caactggacg cttcccggtc agccggataa cacgccgcca ggtggtgaac tggtgtttga
1261 acgctggcgt cggctaagcg ataacagcca gtggattcag gtttcgctgg tcttccagac
1321 tttacagcag atgcgtgata aaacgccgct gtcattaaat acgccgcccg gagaggtgaa
1381 actgaccctg gcaggatgtg aagagcgaaa tgcgcagggc atgtgttcgt tggcaggttt
1441 tacgcaaatc gtgaatgaag cacgcatacc ggcgtgcagt ttgtaatgca taaaaaagag
1501 cattcagtta cctgaatgct ctgaggctga tgacaaacga agaactgtct aatgcgtaga
1561 ccggaaaagg cgttcacgcc gcatccggcc actttcagtt ttcctctttc tcggagtaac
1621 tataaccgta atagttatag ccgtaactgt aagcggtgct ggcgcgttta atcacaccat
1681 tgaggatagc gcctttaata ttgacgcctg cctgttccag acgctgcatt gacaaactca
1741 cctctttggc ggtgttcaag ccaaaacgcg caaccagcag gctggtgcca acagaacgcc
1801 ccacgaccgc ggcatcactc accgccagca tcggcggcgt atcgacaatc accagatcgt
1861 aatggtcgtt cgcccattcc agtaattgac gcatccgatc g

Figure 7b

1 taaggagcag aaacaatgtg gtatttactt tggttcgtcg gcattttgtt gatgtgttcg
61 ctctccaccc ttgtgttggt atggctggac ccgcgtctga aaagttaacg aacgtaggcc
121 tgatgcggcg cattagcatc gcatcaggca atcaataatg tcagatatga aaagcggaaa
181 catatcgatg aaagcgatct taatcccatt tttatctctt ctgattccgt taaccccgca
241 atctgcattc gctcagagtg agccggagct gaagctggaa agtgtggtga ttgtcagtcg
301 tcatggtgtg cgtgctccaa ccaaggccac gcaactgatg caggatgtca ccccagacgc
361 atggccaacc tggccggtaa aactgggttg gctgacaccg cgnggtggtg agctaatcgc
421 ctatctcgga cattaccaac gccagcgtct ggtagccgac ggattgctgg cgaaaaaggg
481 ctgcccgcag tctggtcagg tcgcgattat tgctgatgtc gacgagcgta cccgtaaaac
541 aggcgaagcc ttcgccgccg ggctggcacc tgactgtgca ataaccgtac atacccaggc
601 agatacgtcc agtcccgatc cgttatttaa tcctctaaaa actggcgttt gccaactgga
661 taacgcgaac gtgactgacg cgatcctcag cagggcagga gggtcaattg ctgactttac
721 cgggcatcgg caaacggcgt ttcgcgaact ggaacgggtg cttaattttc cgcaatcaaa
781 cttgtgcctt aaacgtgaga aacaggacga aagctgttca ttaacgcagg cattaccatc
841 ggaactcaag gtgagcgccg acaatgtctc attaaccggt gcggtaagcc tcgcatcaat
901 gctgacggag atatttctcc tgcaacaagc acagggaatg ccggagccgg ggtggggaag
961 gatcaccgat tcacaccagt ggaacacctt gctaagtttg cataacgcgc aattttattt
1021 gctacaacgc acgccagagg ttgcccgcag ccgcgccacc ccgttattag atttgatcaa
1081 gacagcgttg acgccccatc caccgcaaaa acaggcgtat ggtgtgacat tacccacttc
1141 agtgctgttt atcgccggac acgatactaa tctggcaaat ctcggcggcg cactggagct
1201 caactggacg cttcccggtc agccggataa cacgccgcca ggtggtgaac tggtgtttga
1261 acgctggcgt cggctaagcg ataacagcca gtggattcag gtttcgctgg tcttccagac
1321 tttacagcag atgcgtgata aaacgccgct gtcattaaat acgccgcccg gagaggtgaa
1381 actgaccctg gcaggatgtg aagagcgaaa tgcgcagggc atgtgttcgt tggcaggttt
1441 tacgcaaatc gtgaatgaag cacgcatacc ggcgtgcagt ttgtaatgca taaaaaagag
1501 cattcagtta cctgaatgct ctgaggctga tgacaaacga agaactgtct aatgcgtaga
1561 ccggaaaagg cgttcacgcc gcatccggcc actttcagtt ttcctctttc tcggagtaac
1621 tataaccgta atagttatag ccgtaactgt aagcggtgct ggcgcgttta atcacaccat
1681 tgaggatagc gcctttaata ttgacgcctg cctgttccag acgctgcatt gacaaactca
1741 cctctttggc ggtgttcaag ccaaaacgcg caaccagcag gctggtgcca acagaacgcc
1801 ccacgaccgc ggcatcactc accgccagca tcggcggcgt atcgacaatc accagatcgt
1861 aatggtcgtt cgcccattcc agtaattgac gcatccgatc g

Figure 8
Amino acid sequence for E. coli appA (wild type) (SEQ ID NO:8)

MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVT
PDAWPTWPVKLG**W**LTPRGGELIAYLGHY**Q**RQRLVADGLL**A**K**K**GCPQSGQVA
IIADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDNA
NVTDAIL**S**RAGGSIADFTGH**R**QTAFRELERVLNFPQSNLCLKREKQDESCSLTQ
ALPSELKVSAD**N**VSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNT
LLSLHNAQF**Y**LLQRTPEVARSRATPLLDLIKTALTPHPPQKQAYGVTLPTSVLFI
AGHDTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWIQVSL
VFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEARI
PACSL

Bold-Underlined amino acid residues are shown below in the modified appA enzyme (SEQ ID NO:10)

MKAILIPFLSLLIPLTPQSAFAQSEPELKLESVVIVSRHGVRAPTKATQLMQDVT
PDAWPTWPVKLG**E**LTPRGGELIAYLGHY**W**RQRLVADGLL**P**K**C**GCPQSGQVAI
IADVDERTRKTGEAFAAGLAPDCAITVHTQADTSSPDPLFNPLKTGVCQLDNA
NVTDAIL**E**RAGGSIADFTGH**Y**QTAFRELERVLNFPQSNLCLKREKQDESCSLTQ
ALPSELKVSAD**C**VSLTGAVSLASMLTEIFLLQQAQGMPEPGWGRITDSHQWNT
LLSLHNAQF**D**LLQRTPEVARSRATPLLDLIKTALTPHPPQKQAYGVTLPTSVLFI
AGHDTNLANLGGALELNWTLPGQPDNTPPGGELVFERWRRLSDNSQWIQVSL
VFQTLQQMRDKTPLSLNTPPGEVKLTLAGCEERNAQGMCSLAGFTQIVNEARI
PACSL